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L2: Entry 1 of 1 File: USPT Jul 7, 1998

US-PAT-NO: 5776746
DOCUMENT-IDENTIFIER: US 5776746 A

TITLE: Gene amplification methods

DATE-ISSUED: July 7, 1998

INVENTOR-INFORMATION:

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US-CL-CURRENT: 435/464; 435/325, 435/355, 435/356, 435/358

CLAIMS:

I claim:

1. A method for co-amplifying a first recombinant oligonucleotide having a sequence which encodes the amino acid sequence of a protein of interest and a second recombinant oligonucleotide having a sequence encoding an inhibitable enzyme operably linked to a heterologous promoter, comprising:

a) providing:

i) at least one expression vector comprising said first recombinant oligonucleotide having a sequence encoding the amino acid sequence of a protein of interest;

ii) an amplification vector comprising said second recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter; and

iii) a T lymphoid parent cell line;

b) introducing 400 to 500 micrograms of said expression vector and 20 to 30 micrograms of said amplification vector into said parent cell line to generate transformed cells;

c) growing said transformed cells in a first aqueous solution containing an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent growth of said parent cell line; and

d) identifying a transformed cell capable of growth in said first aqueous solution, wherein said transformed cell capable of growth contains an amplified number of copies of said expression vector and an amplified number of copies of said amplification vector.

2. The method of claim 1 wherein said parent cell line contains an endogenous gene encoding a second inhibitable enzyme.

3. The method of claim 2, wherein said second inhibitable enzyme is selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine

deaminase and asparagine synthetase.

4. The method of claim 1 wherein said concentration of inhibitor present in said first aqueous solution is four to six-fold the concentration required to prevent the growth of said parent cell line.

5. The method of claim 2, wherein said first and said second inhibitable enzyme are the same.

6. The method of claim 1 further comprising providing a selection vector encoding a selectable gene product which is introduced into said parent cell line together with said expression vector and said amplification vector.

7. The method of claim 6 wherein said selection vector encodes an active hypoxanthine guanine phosphoribosyltransferase.

8. The method of claim 7 wherein said aqueous solution which requires the expression of said selectable gene product comprises hypoxanthine and azaserine.

9. The method of claim 6 further comprising following the introduction of said vectors the additional step of growing said transformed cell in a second aqueous solution which requires the expression of said selectable gene product prior to growing said transformed cell said first aqueous solution containing an inhibitor capable of inhibiting said inhibitable enzyme.

10. The method of claim 1, wherein said amplification vector encodes an active enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

11. The method of claim 10 wherein said inhibitor is selected from the group consisting of methotrexate, 2'-deoxycoformycin, methionine sulphoximine, albizziin and .beta.-aspartyl hydroxamate.

12. A method, comprising:

a) providing:

i) at least one expression vector comprising a first recombinant oligonucleotide having a sequence encoding the amino acid sequence of a protein of interest;

ii) an amplification vector comprising a second recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter; and

iii) a T lymphoid parent cell line;

b) introducing said expression vector and said amplification vector into said parent cell line to generate transformed cells;

c) introducing said transformed cells into a first aqueous solution, said first aqueous solution comprising an inhibitor capable of inhibiting said first inhibitable enzyme and wherein the concentration of said inhibitor present in said first aqueous solution is four-fold to six-fold the concentration of said inhibitor required to prevent the growth of said parent cell line; and

d) identifying at least one transformed cell capable of growth in said first aqueous solution, wherein said transformed cell capable of growth contains an amplified number of copies of said expression vector and an amplified number of copies of said amplification vector.

13. The method of claim 12, wherein said parent cell line contains an endogenous gene encoding a second inhibitable enzyme.

14. The method of claim 13, wherein said second inhibitable enzyme is selected from the group consisting of dihydrofolate reductase, glutamine synthetase,

adenosine deaminase and asparagine synthetase.

15. The method of claim 13, wherein said first and said second inhibitable enzyme are the same.

16. The method of claim 12, further comprising providing a selection vector encoding a selectable gene product which is introduced into said parent cell line together with said expression vector and said amplification vector.

17. The method of claim 16, wherein said selection vector encodes an active enzyme selected from the group comprising hypoxanthine guanine phosphoribosyltransferase, hygromycin G phosphotransferase, xanthine-guanine phosphoribosyltransferase and aminoglycoside 3' phosphotransferase.

18. The method of claim 17, wherein said selection vector encodes an active hypoxanthine guanine phosphoribosyltransferase.

19. The method of claim 18, wherein said first aqueous solution further comprises hypoxanthine and azaserine.

20. The method of claim 16, further comprising, following the introduction of said expression, amplification and selection vectors, the additional step of introducing said transformed cells into a second aqueous solution, said second aqueous solution requiring the expression of said selectable gene product for growth of said transformed cells, prior to introducing said transformed cells into said first aqueous solution.

21. The method of claim 12, wherein said amplification vector encodes an active enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

22. The method of claim 21, wherein said inhibitor is selected from the group consisting of methotrexate, 2'-deoxycoformycin, methionine sulphoximine, albizziin and .beta.-aspartyl hydroxamate.

23. The method of claim 12 further comprising the steps of:

e) introducing said transformed cell capable of growth in said first aqueous solution into a second aqueous solution, said second aqueous solution comprising said inhibitor capable of inhibiting said first inhibitable enzyme and wherein the concentration of said inhibitor present in said second aqueous solution is sixteen-fold to thirty-six-fold the concentration of said inhibitor required to prevent the growth of said parent cell line; and

f) identifying at least one transformed cell capable of growth in said second aqueous solution.

24. The method of claim 12, wherein 20 to 30 micrograms of said amplification vector and a total of 400 to 500 micrograms of said expression vector are introduced into said parent cell line.

25. The method of claim 16, wherein 10 to 15 micrograms of said selection vector, 20 to 30 micrograms of said amplification vector and a total of 400 to 500 micrograms of said expression vector are introduced into said parent cell line.

26. The method of claim 12, wherein said expression and amplification vectors are linearized prior to introduction into said parent cell line.

27. The method of claim 12, wherein said T lymphoid cell line is the BW5147.G.1.4 cell line.

28. A method, comprising:

a) providing:

i) at least one expression vector comprising a first recombinant oligonucleotide having a sequence encoding the amino acid sequence of a protein of interest;

ii) an amplification vector comprising a second recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter;

iii) a selection vector comprising a third recombinant oligonucleotide having a sequence which encodes a selectable gene product; and

iv) a T lymphoid parent cell line;

b) introducing said expression vector, said amplification vector and said selection vector into said cell line to generate transformed cells;

c) introducing said transformed cells into a first aqueous solution, said first aqueous solution requiring the expression of said selectable gene product for growth of said transformed cells;

d) identifying at least one transformed cell capable of growth in said first aqueous solution;

e) introducing said transformed cell capable of growth in said first aqueous solution into a second aqueous solution, said second aqueous solution comprising an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent growth of said parent cell line; and

f) identifying at least one transformed cell capable of growth in said second aqueous solution, wherein said transformed cell capable of growth contains an amplified number of copies of said expression vector and an amplified number of copies of said amplification vector.

29. The method of claim 28, wherein said parent cell line contains an endogenous gene encoding a second inhibitable enzyme.

30. The method of claim 29, wherein said second inhibitable enzyme is selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

31. The method of claim 29, wherein said first and said second inhibitable enzyme are the same.

32. The method of claim 28, wherein 10 to 15 micrograms of said selection vector, 20 to 30 micrograms of said amplification vector and a total of 400 to 500 micrograms of said at least one expression vector are introduced into said parent cell line.

33. The method of claim 28, wherein said concentration of inhibitor present in said second aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said parent cell line.

34. The method of claim 33 further comprising the steps of:

g) introducing said transformed cell capable of growth in said first aqueous solution into a third aqueous solution, said third aqueous solution comprising said inhibitor capable of inhibiting said first inhibitable enzyme and wherein the concentration of said inhibitor present in said third aqueous solution is sixteen-fold to thirty-six-fold the concentration of said inhibitor required to prevent the growth of said parent cell line; and

h) identifying at least one transformed cell capable of growth in said third aqueous solution.

35. The method of claim 28, wherein said selection vector encodes an active enzyme

selected from the group comprising hypoxanthine guanine phosphoribosyltransferase, hygromycin G phosphotransferase, xanthine-guanine phosphoribosyltransferase and aminoglycoside 3' phosphotransferase.

36. The method of claim 28, wherein said T lymphoid cell line is the BW5147.G.1.4 cell line.

37. The method of claim 28, wherein said amplification vector encodes an active enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase, asparagine synthetase.

38. The method of claim 37, wherein said inhibitor is selected from the group consisting of methotrexate, 2'-deoxycoformycin, methionine sulphoximine, albizziin and .beta.-aspartyl hydroxamate.

39. The method of claim 28, wherein said expression, amplification and selection vectors are linearized prior to introduction into said parent cell line.

40. A method, comprising:

a) providing:

i) at least one expression vector comprising a first recombinant oligonucleotide having a sequence encoding the amino acid sequence of a protein of interest;

ii) an amplification vector comprising a second recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter;

iii) a selection vector comprising a third recombinant oligonucleotide having a sequence which encodes a selectable gene product; and

iv) a T lymphoid parent cell line;

b) introducing said expression vector, said amplification vector and said selection vector into said cell line to generate transformed cells;

c) introducing said transformed cells into a first aqueous solution, said first aqueous solution requiring the expression of said selectable gene product for growth of said transformed cells;

d) identifying at least one individual clone of transformed cells capable of growth in said first aqueous solution;

e) introducing said individual clone capable of growth in said first aqueous solution into a second aqueous solution, said second aqueous solution comprising an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent growth of said parent cell line; and

f) identifying at least one individual clone capable of growth in said second aqueous solution, wherein said clone capable of growth contains an amplified number of copies of said expression vector and an amplified number of copies of said amplification vector.

41. The method of claim 40, wherein said parent cell line contains an endogenous gene encoding a second inhibitable enzyme.

42. The method of claim 41, wherein said second inhibitable enzyme is selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

43. The method of claim 41, wherein said first and said second inhibitable enzyme are the same.

44. The method of claim 40, wherein 10 to 15 micrograms of said selection vector, 20 to 30 micrograms of said amplification vector and a total of 400 to 500 micrograms of said at least one expression vector are introduced into said parent cell line.

45. The method of claim 40, wherein said concentration of inhibitor present in said second aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said parent cell line.

46. The method of claim 45 further comprising the steps of:

g) introducing said transformed cell capable of growth in said first aqueous solution into a third aqueous solution, said third aqueous solution comprising said inhibitor capable of inhibiting said first inhibitable enzyme and wherein the concentration of said inhibitor present in said third aqueous solution is sixteen-fold to thirty-six-fold the concentration of said inhibitor required to prevent the growth of said parent cell line; and

h) identifying at least one transformed cell capable of growth in said third aqueous solution.

47. The method of claim 40, wherein said selection vector encodes an active enzyme selected from the group comprising hypoxanthine guanine phosphoribosyltransferase, hygromycin G phosphotransferase, xanthine-guanine phosphoribosyltransferase and aminoglycoside 3' phosphotransferase.

48. The method of claim 40, wherein said T lymphoid cell line is the BW5147.G.1.4 cell line.

49. The method of claim 40, wherein said amplification vector encodes an active enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

50. The method of claim 49, wherein said inhibitor is selected from the group consisting of methotrexate, 2'-deoxycoformycin, methionine sulphoximine, albizziin and .beta.-aspartyl hydroxamate.

51. The method of claim 49, wherein said T lymphoid cell line is the BW5147.G.1.4 cell line.

52. The method of claim 40, wherein said expression, amplification and selection vectors are linearized prior to introduction into said parent cell line.

53. A method, comprising:

a) providing:

i) at least one expression vector comprising a first recombinant oligonucleotide having a sequence encoding the amino acid sequence of a protein of interest;

ii) an amplification vector comprising a second recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter; and

iii) a T lymphoid parent cell line;

b) introducing said expression vector and said amplification vector into said T lymphoid parent cell line to generate transformed cells;

c) introducing said transformed cells into a first aqueous solution, said first aqueous solution comprising an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent the growth of said parent cell line; and

d) identifying a transformed cell capable of growth in said first aqueous solution, wherein said transformed cell capable of growth contains an amplified number of copies of said expression vector and an amplified number of copies of said amplification vector.

54. The method of claim 53, wherein said parent cell line contains an endogenous gene encoding a second inhibitable enzyme.

55. The method of claim 54, wherein said second inhabitable enzyme is selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

56. The method of claim 54, wherein said first and said second inhibitable enzyme are the same.

57. The method of claim 53, wherein said concentration of inhibitor present in said first aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said parent cell line, and said method further comprising the steps of:

e) introducing said transformed cell capable of growth in said first aqueous solution into a second aqueous solution, said second aqueous solution comprising said inhibitor capable of inhibiting said first inhibitable enzyme and wherein the concentration of said inhibitor present in said second aqueous solution is sixteen-fold to thirty-six-fold the concentration of said inhibitor required to prevent the growth of said parent cell line; and

f) identifying at least one transformed cell capable of growth in said second aqueous solution.

58. The method of claim 53 further comprising providing a selection vector encoding a selectable gene product which is introduced into said parent cell line together with said expression vector and said amplification vector.

59. The method of claim 58, wherein said selection vector encodes an active enzyme selected from the group comprising hypoxanthine guanine phosphoribosyltransferase, hygromycin G phosphotransferase, xanthine-guanine phosphoribosyltransferase and aminoglycoside 3' phosphotransferase.

60. The method of claim 55, further comprising following the introduction of said expression, amplification and selection vectors the additional step of introducing said transformed cells into a second aqueous solution, said second aqueous solution requiring the expression of said selectable gene product for growth of said transformed cells, prior to introducing said transformed cells into said first aqueous solution.

61. The method of claim 53, wherein said amplification vector encodes an active enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

62. The method of claim 61, wherein said inhibitor is selected from the group consisting of methotrexate, 2'-deoxycoformycin, methionine sulphoximine, albizziin and .beta.-aspartyl hydroxamate.

63. The method of claim 53, wherein 20 to 30 micrograms of said amplification vector and a total of 400 to 500 micrograms of said expression vector are introduced into said parent cell line.

64. The method of claim 58, wherein 10 to 15 micrograms of said selection vector, 20 to 30 micrograms of said amplification vector and a total of 400 to 500 micrograms of said expression vector are introduced into said parent cell line.

65. The method of claim 53, wherein said expression and amplification vectors are linearized prior to introduction into said parent cell line.

66. A method, comprising:

a) providing:

i) at least one expression vector comprising a first recombinant oligonucleotide having a sequence encoding the amino acid sequence of a protein of interest;

ii) an amplification vector comprising a second recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter; and

iii) a T lymphoid parent cell line;

b) treating said expression vector and said amplification vector with a restriction enzyme to create a linearized expression vector and a linearized amplification vector;

c) introducing said linearized expression vector and said linearized amplification vector into said parent cell line to generate transformed cells;

d) introducing said transformed cells into a first aqueous solution, said first aqueous solution comprising an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent growth of said parent cell line; and

e) identifying a transformed cell capable of growth in said first aqueous solution, wherein said transformed cell capable of growth contains an amplified number of copies of said expression vector and an amplified number of copies of said amplification vector.

67. The method of claim 66, wherein said T lymphoid cell line is the BW5147.G.1.4 cell line.

68. The method of claim 66, wherein said parent cell line contains an endogenous gene encoding a second inhibitable enzyme.

69. The method of claim 68, wherein said second inhibitable enzyme is selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

70. The method of claim 66, wherein said concentration of inhibitor present in said first aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said parent cell line.

71. The method of claim 68, wherein said first and said second inhibitable enzyme are the same.

72. The method of claim 66 further comprising providing a selection vector encoding a selectable gene product which is introduced into said parent cell line together with said expression vector and said amplification vector.

73. The method of claim 72, wherein said selection vector encodes an active enzyme selected from the group comprising hypoxanthine guanine phosphoribosyltransferase, hygromycin G phosphotransferase, xanthine-guanine phosphoribosyltransferase and aminoglycoside 3' phosphotransferase.

74. The method of claim 72 further comprising, following the introduction of said selection, expression and amplification vectors, the additional step of introducing said transformed cells into a second aqueous solution, said second aqueous solution requiring the expression of said selectable gene product for growth of said transformed cells, prior to introducing said transformed cell into said first aqueous solution.

75. The method of claim 66, wherein said amplification vector encodes an active

enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminated and asparagine synthetase.

76. The method of claim 75, wherein said inhibitor is selected from the group consisting of methotrexate, 2'-deoxycoformycin, methionine sulphoximine, albizziin and .beta.-aspartyl hydroxamate.

77. The method of claim 70 further comprising the steps of:

f) introducing said transformed cell capable of growth in said first aqueous solution into a second aqueous solution, said second aqueous solution comprising said inhibitor capable of inhibiting said first inhibitable enzyme and wherein the concentration of said inhibitor present in said second aqueous solution is sixteen-fold to thirty-six fold the concentration of said inhibitor required to prevent the growth of said parent cell line; and

g) identifying at least one transformed cell capable of growth in said second aqueous solution.

78. The method of claim 66, wherein 20 to 30 micrograms of said amplification vector and a total of 400 to 500 micrograms of said expression vector are introduced into said parent cell line.

79. The method of claim 72, wherein 10 to 15 micrograms of said selection vector, 20 to 30 micrograms of said amplification vector and a total of 400 to 500 micrograms of said expression vector are introduced into said parent cell line.

80. The method of claim 71, wherein said expression and amplification vectors are linearized prior to introduction into said parent cell line.

81. The method of claim 76 further comprising providing a selection vector encoding a selectable gene product which is introduced into said parent cell line together with said expression vector and said amplification vector.

82. The method of claim 81, wherein said selection vector encodes an active enzyme selected from the group comprising hypoxanthine guanine phosphoribosyltransferase, hygromycin G phosphotransferase, xanthine-guanine phosphoribosyltransferase and aminoglycoside 3' phosphotransferase.

83. The method of claim 81, further comprising, following the introduction of said vector comprising said first and second recombinant oligonucleotides, the additional step of introducing said transformed cells into a third aqueous solution, said third aqueous solution requiring the expression of said selectable gene product for growth of said transformed cells, prior to introducing said transformed cells into said first aqueous solution.

84. A method, comprising:

a) providing:

i) a vector comprising a first recombinant oligonucleotide having a sequence encoding the amino acid sequence of a protein of interest and a second recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter; and

ii) a T lymphoid parent cell line;

b) introducing said vector into said T lymphoid parent cell line to generate transformed cells;

c) introducing said transformed cells into a first aqueous solution, said first aqueous solution comprising an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent the growth of said parent cell line; and

d) identifying a transformed cell capable of growth in said first aqueous solution, wherein said transformed cell capable of growth contains an amplified number of copies of said vector.

85. The method of claim 84, wherein said parent cell line contains an endogenous gene encoding a second inhibitable enzyme.

86. The method of claim 85, wherein said second inhibitable enzyme is selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

87. The method of claim 85, wherein said first and said second inhibitable enzyme are the same.

88. The method of claim 84, wherein said concentration of inhibitor present in said first aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said parent cell line.

89. The method of claim 88 further comprising the steps of:

e) introducing said transformed cell capable of growth in said first aqueous solution into a second aqueous solution, said second aqueous solution comprising said inhibitor capable of inhibiting said first inhibitable enzyme and wherein the concentration of said inhibitor present in said second aqueous solution is sixteen-fold to thirty-six-fold the concentration of said inhibitor required to prevent the growth of said parent cell line; and

f) identifying at least one transformed cell capable of growth in said second aqueous solution.

90. The method of claim 84, wherein said second recombinant oligonucleotide encodes an active enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

91. The method of claim 90, wherein said inhibitor is selected from the group consisting of methotrexate, 2'-deoxycoformycin, methionine sulphoximine, albizziin and .beta.-aspartyl hydroxamate.

92. The method of claim 84, wherein said T lymphoid cell line is the BW5147.G.1.4 cell line.

93. The method of claim 84, wherein said vector is linearized prior to introduction into said parent cell line.

94. A composition comprising a T lymphoid cell line having an amplified gene said gene amplified from a plurality of exogenous integrated nucleic acid, said nucleic acid comprising a recombinant oligonucleotide having a sequence encoding an inhibitable enzyme operably linked to a heterologous promoter.

95. The composition of claim 94, wherein said recombinant oligonucleotide having a sequence encoding an inhibitable enzyme encodes an active dihydrofolate reductase.

96. The composition of claim 94, wherein said parent T lymphoid cell line is the BW5147.G.1.4 cell line.

97. The composition of claim 94 further comprising an integrated recombinant oligonucleotide comprising a gene encoding a protein of interest.

98. The composition of claim 94 further comprising an integrated recombinant oligonucleotide comprising a gene encoding a selectable marker.

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L1: Entry 1 of 1 File: USPT Oct 26, 1999

US-PAT-NO: 5972334
DOCUMENT-IDENTIFIER: US 5972334 A

TITLE: Vaccines for treatment of lymphoma and leukemia

DATE-ISSUED: October 26, 1999

INVENTOR-INFORMATION:

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US-CL-CURRENT: 424/131.1; 424/141.1, 435/320.1, 435/325, 435/326, 435/327, 435/343.1, 435/372.3, 435/68.1, 435/69.7, 530/387.2, 536/23.53

CLAIMS:

What is claimed is:

1. A method of producing a vaccine for treatment of B-cell lymphoma comprising:

a) providing:

i) malignant B cells isolated from a patient having a B-cell lymphoma;

ii) an expression vector;

iii) an amplification vector comprising a recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter; and

iv) a T lymphoid parent cell line;

b) isolating nucleic acid from said malignant cells, said nucleic acid comprising nucleotide sequences encoding at least one V.sub.H region and at least one V.sub.L region, said V.sub.H and V.sub.L regions derived from immunoglobulin molecules expressed by said malignant cells;

c) inserting said nucleotide sequences encoding said V.sub.H and V.sub.L regions into said expression vector;

d) introducing said expression vector and said amplification vector into said parent cell line to generate one or more transformed cells;

e) growing said transformed cells in a first aqueous solution containing an inhibitor capable of inhibiting said first inhibitable enzyme wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent growth of said parent cell line; and

f) identifying a transformed cell capable of growth in said first aqueous solution, wherein said transformed cell capable of growth expresses said V.sub.H and V.sub.L regions wherein V.sub.H and V.sub.L regions comprise a protein molecule useful as said vaccine.

2. The methods of claim 1, wherein transformed cell capable of growth contains an amplified number of copies of said expression vector and an amplified number of copies of said amplification vector.

3. The method of claim 1, wherein nucleotide sequences encoding said V.sub.H and V.sub.L regions comprise at least two V.sub.H and at least two V.sub.L regions.

4. The method of claim 1, wherein said T lymphoid parent cell line is the BW5147.G.1.4 cell line.

5. The method of claim 1, wherein said parent cell line contains an endogenous gene encoding a second inhibitable enzyme.

6. The method of claim 5, wherein said second inhibitable enzyme is selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase, and asparagine synthetase.

7. The method of claim 1, wherein said concentration of inhibitor present in said first aqueous solution is four to six-fold the concentration required to prevent the growth of said parent cell line.

8. The method of claim 5, wherein said first and said second inhibitable enzyme are the same.

9. The method of claim 1, further comprising providing a selection vector encoding a selectable gene product which is introduced into said parent cell line together with said expression vector and said amplification vector.

10. The method of claim 9, wherein said selection vector encodes an active hypoxanthine guanine phosphoribosyltransferase.

11. The method of claim 9, further comprising, following the introduction of said vectors, the additional step of growing said transformed cell in a second aqueous solution which requires the expression of said selectable gene product prior to growing said transformed cells in said first aqueous solution containing an inhibitor capable of inhibiting said first inhibitable enzyme.

12. The method of claim 11, wherein said second aqueous solution comprises hypoxanthine and azaserine.

13. The method of claim 1, wherein said inhibitable enzyme encoded by said amplification vector is an active enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase, and asparagine synthetase.

14. The method of claim 13, wherein said inhibitor is selected from the group consisting of methotrexate, 2'-deoxycoformycin, methionine sulphoximine, albizziin and .beta.-aspartyl hydroxamate.

15. The method of claim 1, wherein between approximately 20 and 30 micrograms of said amplification vector and between approximately 400 and 500 micrograms of said expression vector are introduced into said parent cell line.

16. A method of producing a vaccine for treatment of B cell lymphoma, comprising:

a) providing:

i) malignant B cells isolated from a patient having a B-cell lymphoma;

ii) an expression vector;

iii) an amplification vector comprising a first recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter;

iv) a selection vector comprising a second recombinant oligonucleotide having a sequence which encodes a selectable gene product; and

v) a T lymphoid parent cell line;

b) isolating nucleic acid from said malignant cells, said nucleic acid comprising nucleotide sequences encoding at least one V.sub.H region and at least one V.sub.L region, said V.sub.H and V.sub.L regions derived from immunoglobulin molecules expressed by said malignant cells;

c) inserting said nucleotide sequences encoding said V.sub.H and V.sub.L regions into said expression vector;

d) introducing said expression vector, said amplification vector and said selection vector into said parent cell line to generate transformed cells;

e) introducing said transformed cells into a first aqueous solution, said first aqueous solution requiring the expression of said selectable gene product for growth of said transformed cells;

f) identifying at least one transformed cell capable of growth in said first aqueous solution;

g) introducing said transformed cell capable of growth in said first aqueous medium into a second aqueous solution, said second aqueous solution comprising an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said second aqueous solution is sufficient to prevent growth of said parent cell line; and

h) identifying at least one transformed cell capable of growth in said second aqueous solution, wherein said transformed cell capable of growth expresses said V.sub.H and V.sub.L regions wherein said V.sub.H and V.sub.L regions comprise a protein molecule.

17. The method of claim 16, wherein said parent cell line contains an endogenous gene encoding a second inhibitable enzyme.

18. The method of claim 17, wherein said second inhibitable enzyme is selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

19. The method of claim 17, wherein said first and said second inhibitable enzyme are the same.

20. The method of claim 16, wherein between approximately 10 and 15 micrograms of said selection vector, between approximately 20 and 30 micrograms of said amplification vector and between approximately 400 and 500 micrograms of said expression vector are introduced into said parent cell line.

21. The method of claim 16, wherein said concentration of inhibitor present in said second aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said parent cell line.

22. The method of claim 21 further comprising the steps of:

i) introducing said transformed cell capable of growth in said second aqueous solution into a third aqueous solution, said third aqueous solution comprising said inhibitor capable of inhibiting said first inhibitable enzyme and wherein the concentration of said inhibitor present in said third aqueous solution is sixteen-fold to thirty-six-fold the concentration of said inhibitor required to prevent the growth of said parent cell line; and

j) identifying at least one transformed cell capable of growth in said third aqueous solution.

23. The method of claim 16, wherein said selection vector encodes an active enzyme selected from the group comprising hypoxanthine guanine phosphoribosyltransferase, hygromycin G phosphotransferase, xanthine-guanine phosphoribosyltransferase and aminoglycoside 3' phosphotransferase.

24. The method of claim 16, wherein said T lymphoid cell line is the BW5147.G.1.4 cell line.

25. The method of claim 16, wherein said inhibitable enzyme encoded by said amplification vector is an active enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase, asparagine synthetase.

26. The method of claim 25, wherein said inhibitor is selected from the group consisting of methotrexate, 2'-deoxycoformycin, methionine sulphoximine, albizziin and .beta.-aspartyl hydroxamate.

27. The method of claim 16, wherein said expression, amplification and selection vectors are linearized prior to introduction into said parent cell line.

28. A method of producing a vaccine for treatment of B cell lymphoma, comprising:

a) providing:

i) malignant B cells isolated from a patient having a B-cell lymphoma;

ii) an expression vector;

iii) an amplification vector comprising a first recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter;

iv) a selection vector comprising a second recombinant oligonucleotide having a sequence which encodes a selectable gene product; and

v) a T lymphoid parent cell line;

b) isolating nucleic acid from said malignant cells, said nucleic acid comprising nucleotide sequences encoding at least one V.sub.H region and at least one V.sub.L region, said V.sub.H and V.sub.L regions derived from immunoglobulin molecules expressed by said malignant cells;

c) inserting said nucleotide sequences encoding said V.sub.H and V.sub.L regions into said expression vector;

d) introducing said expression vector, said amplification vector and said selection vector into said parent cell line to generate transformed cells;

e) introducing said transformed cells into a first aqueous solution, said first aqueous solution requiring the expression of said selectable gene product for growth of said transformed cells;

f) identifying at least one individual clone of transformed cells capable of growth in said first aqueous solution;

g) introducing said individual clone capable of growth in said first aqueous solution into a second aqueous solution, said second aqueous solution comprising an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent growth of said parent cell line; and

h) identifying at least one individual clone capable of growth in said second aqueous solution, wherein said clone capable of growth expresses said V.sub.H and V.sub.L regions wherein said V.sub.H and V.sub.L regions comprise a protein molecule.

29. The method of claim 28, wherein said parent cell line contains an endogenous gene encoding a second inhibitable enzyme.

30. The method of claim 29, wherein said second inhibitable enzyme is selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

31. The method of claim 29, wherein said first and said second inhibitable enzyme are the same.

32. The method of claim 28, wherein between approximately 10 and 15 micrograms of said selection vector, between approximately 20 and 30 micrograms of said amplification vector and between approximately 400 and 500 micrograms of said expression vector are introduced into said parent cell line.

33. The method of claim 28, wherein said concentration of inhibitor present in said second aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said parent cell line.

34. The method of claim 33 further comprising the steps of:

i) introducing said transformed cell capable of growth in said second aqueous solution into a third aqueous solution, said third aqueous solution comprising said inhibitor capable of inhibiting said first inhibitable enzyme and wherein the concentration of said inhibitor present in said third aqueous solution is sixteen-fold to thirty-six-fold the concentration of said inhibitor required to prevent the growth of said parent cell line; and

j) identifying at least one transformed cell capable of growth in said third aqueous solution.

35. The method of claim 28, wherein said selection vector encodes an active enzyme selected from the group comprising hypoxanthine guanine phosphoribosyltransferase, hygromycin G phosphotransferase, xanthine-guanine phosphoribosyltransferase and aminoglycoside 3' phosphotransferase.

36. The method of claim 28, wherein said T lymphoid parent cell line is the BW5147.G.1.4 cell line.

37. The method of claim 28, wherein said inhibitable enzyme encoded by said amplification vector is an active enzyme selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

38. The method of claim 27, wherein said inhibitor is selected from the group consisting of methotrexate, 2'-deoxycoformycin, methionine sulphoximine, albizziin and .beta.-aspartyl hydroxamate.

39. The method of claim 28, wherein said expression, amplification and selection vectors are linearized prior to introduction into said parent cell line.

40. A method of amplifying the number of copies of a vector, comprising:

a) providing:

i) a vector comprising a first recombinant oligonucleotide comprising nucleotide sequences encoding at least one V.sub.H region and at least one V.sub.L region, said V.sub.H and V.sub.L regions derived from immunoglobulin molecules expressed by malignant B cells isolated from a patient having a B-cell lymphoma and a second recombinant oligonucleotide having a sequence encoding a first inhibitable enzyme operably linked to a heterologous promoter; and

ii) a T lymphoid parent cell line;

b) introducing said vector into said T lymphoid parent cell line to generate transformed cells;

c) introducing said transformed cells into a first aqueous solution, said first aqueous solution comprising an inhibitor capable of inhibiting said first inhibitable enzyme, wherein the concentration of said inhibitor present in said first aqueous solution is sufficient to prevent the growth of said parent cell line; and

d) identifying a transformed cell capable of growth in said first aqueous solution, wherein said transformed cell capable of growth contains an amplified number of copies of said vector.

41. The method of claim 40, wherein said parent cell line contains an endogenous gene encoding a second inhibitable enzyme.

42. The method of claim 41, wherein said second inhibitable enzyme is selected from the group consisting of dihydrofolate reductase, glutamine synthetase, adenosine deaminase and asparagine synthetase.

43. The method of claim 41, wherein said first and said second inhibitable enzyme are the same.

44. The method of claim 40, wherein said concentration of inhibitor present in said second aqueous solution is four-fold to six-fold the concentration required to prevent the growth of said parent cell line.

45. The method of claim 44 further comprising the steps of:

e) introducing said transformed cell capable of growth in said first aqueous solution into a second aqueous solution, said second aqueous solution comprising said inhibitor capable of inhibiting said first inhibitable enzyme and wherein the concentration of said inhibitor present in said second aqueous solution is sixteen-fold to thirty-six-fold the concentration of said inhibitor required to prevent the growth of said parent cell line; and

f) identifying at least one transformed cell capable of growth in said second aqueous solution.

46. The method of claim 40 further comprising providing a selection vector encoding a selectable gene product which is introduced into said parent cell line together with said vector comprising said first and second recombinant oligonucleotides.

47. The method of claim 40, wherein said vector is linearized prior to introduction into said parent cell line.

L16 ANSWER 17 OF 41 CANCERLIT
ACCESSION NUMBER: 97614217 CANCERLIT
DOCUMENT NUMBER: 97614217
TITLE: Monoclonal antibodies as agonists: an expanded role for their use in cancer therapy (Meeting abstract).
AUTHOR: Vitetta E S; Racila E; Marches R; Tucker T; Scheuermann R; Uhr J W
CORPORATE SOURCE: University of Texas Southwestern Medical Center, Dallas, TX.
SOURCE: Can J Infect, (1995) 6 (Suppl C) 283C.
DOCUMENT TYPE: (MEETING ABSTRACTS)
LANGUAGE: English
FILE SEGMENT: Institute for Cell and Developmental Biology
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 19980417
Last Updated on STN: 19980417

AB F(ab')2 fragments of MAbs directed against CD19 or the idiotype or isotype of membrane Ig on B **lymphoma cells** have induced anti-tumor effects in both syngeneic (BCL1) and xenogeneic (SCID/Daudi) models of B lymphoma. These (IgG) MAbs, induce cell cycle arrest (CCA) and/or apoptosis in B **lymphoma cells** in vitro even in the absence of T cells and the continued administration to mice induces a dormant tumor state. In combination with chemotherapy or ITS, such MAbs are curative. The induction of CCA and apoptosis is dependent upon the affinity of the MAb, the epitope recognized, and the extent of cross-linking of the target cell surface molecule. There appears to be a bifurcation in the signalling pathways leading to CCA and apoptosis since antisense oligonucleotides targeted at the lyn tyrosine kinase gene inhibit CCA but not apoptosis.

L13 ANSWER 37 OF 40 CANCERLIT
ACCESSION NUMBER: 96606822 CANCERLIT
DOCUMENT NUMBER: 96606822
TITLE: Idiotypic DNA **vaccines** against **B-cell lymphoma** (Meeting abstract).
AUTHOR: Stevenson F K; Zhu D; Ashworth L J; King C A; Hawkins R E
CORPORATE SOURCE: Molecular Immunology Group, Tenovus Laboratory, Southampton University Hospitals, Southampton, UK.
SOURCE: Non-serial, (1995) Gene Therapy of Cancer: 2nd European Conference, September 7-8, 1995, London, A14, 1995 .
DOCUMENT TYPE: (MEETING ABSTRACTS)
LANGUAGE: English
FILE SEGMENT: Institute for Cell and Developmental Biology
ENTRY MONTH: 199605
ENTRY DATE: Entered STN: 19970509
Last Updated on STN: 19970509

AB Idiotypic determinants provide clearly defined tumor-associated protein antigens, which can induce protective immunity against **B-cell lymphoma**. Because each patient requires an individual **vaccine**, idiotypic antigens are also ideal candidates for exploring the feasibility of replacing protein antigens by DNA **vaccines**. Variable region gene sequences which encode idiotypic determinants have been identified from 54 patients' tumor biopsies with a success rate of 90%. In order to create **vaccines**, amplified products from the model murine lymphoma, BCL1, and from selected patients with lymphoma have been assembled as single chain (sc) Fv sequences. Recombinant scFv protein has been expressed in vitro by bacteria or by mammalian cells. To explore vaccination via direct transfection of naked DNA in vivo, plasmids incorporating scFv DNA from the BCL1 lymphoma have been injected into mouse muscle. Low levels of serum anti-idiotypic antibody, and splenic T cells which proliferate in response to idiotypic protein have been induced. These results have led to a small clinical trial of scFv DNA vaccination in patients with advanced low-grade lymphoma, and two patients have been treated so far. The mouse BCL1 model is being used to optimize **vaccine** design and delivery. Co-injection of cytokine-encoding vectors (IL-2 and GM-CSF) with the scFv vector has been found to promote the anti-idiotypic response. To fully realize the potential of cytokine manipulation of immunity, methods to regulate both cytokine and scFv gene expression are required, and these are being developed.

L13 ANSWER 40 OF 40 CANCERLIT
ACCESSION NUMBER: 96605256 CANCERLIT
DOCUMENT NUMBER: 96605256
TITLE: Applications of antibody gene technology (Meeting abstract).
AUTHOR: Hawkins R E
CORPORATE SOURCE: CRC Dept. of Clinical Oncology, Hills Road, Cambridge CB2 2Q, UK.
SOURCE: Br J Cancer, (1994) 71 (Suppl 24) 1.
ISSN: 0007-0920.
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Institute for Cell and Developmental Biology
ENTRY MONTH: 199605
ENTRY DATE: Entered STN: 19970509
Last Updated on STN: 19970509

AB Recent developments in antibody technology are enabling new therapeutic approaches to be investigated. The ability to work with antibody genes rather than protein molecules is an enormous advantage. We are exploring two major applications of this technology, one using antibody molecules as therapeutic targets and the other new methods of making antibodies. The use of the polymerase chain reaction to rapidly clone genes followed by DNA sequencing enables us to rapidly identify tumor-derived V-genes from lymph node biopsies of patients with **B-cell lymphoma**. Both the VH and VL genes have been identified in 11/13 patients. This is a true tumor specific antigen and is thus a potential candidate for antitumor vaccination. However, there is the complication that the **vaccine** must be made individually for each patient. Plasmid vaccination appears to offer the ideal solution to test anti-idiotypic vaccination as a therapeutic approach. In mice we can generate antibody and T-cell responses to the idiotypic antigens and a phase I trial is underway. Enhanced immune responses can be obtained by incorporating other immunostimulatory molecules such as GM-CSF or IL2 into the vectors. These molecules increase both the antibody and T-cell responses but, in view of the potential toxicity, may require control of gene expression. Other potential target antigens for antitumor vaccination are being explored. The cloning of repertoires of V-genes and their display on the surface of bacteriophage allows large libraries of antibody fragments to be made. Antibodies with the desired binding characteristics can be selected directly. The same approach can also be used to improve existing antibodies. The antibodies made can be human or murine depending on the starting material. New developments in the technology allow very large libraries (approx 10(11)) to be made and promise to allow the rapid isolation of human antibodies with good affinity directly. Already, antibodies made from phage display libraries are being tested in imaging trials and many ways of endowing these binding molecules with effector mechanisms are being investigated. The ease of manipulating antibody genes facilitates the construction of fusion proteins and of bispecific forms with anticancer potential. Furthermore, gene therapy approaches to allow targeted delivery and expression of antibody fragments allows prospects of producing antibody fragments locally within tumors. This approach potentially enhances their specificity and by continued local production may enhance the effectiveness of antibody based therapy.

L13 ANSWER 14 OF 40 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2002383302 MEDLINE
DOCUMENT NUMBER: 22127460 PubMed ID: 12126549
TITLE: Specific humoral immune response against **B-cell lymphoma** elicited by mixed **immunoglobulin fragments**.
AUTHOR: Lin Ningjing; Zhu Ping; Zhang Xin; Dong Yujun; Ren Yali; Wang Yijia; Li Wanqing
CORPORATE SOURCE: Department of Hematology, First Hospital of Peking University, Beijing, China.
SOURCE: CHUNG-HUA I HSUEH TSA CHIH [CHINESE MEDICAL JOURNAL], (2002 Jun 10) 82 (11) 766-70.
Journal code: 7511141. ISSN: 0376-2491.
PUB. COUNTRY: China
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Chinese
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200208
ENTRY DATE: Entered STN: 20020720
Last Updated on STN: 20020830
Entered Medline: 20020829

AB OBJECTIVE: To explore the feasibility of construction of the universal nucleic acid **vaccine** against **B-cell lymphoma**. METHODS: RT-PCR was employed to obtain the immuncy losulin heavy chain variable region (IgHV1) gene fragments of the Namalwa cell, normal fetal umbilical cord blood and adult peripheral blood. Then these RT-PCR products were cloned into the eukaryotic expression vector pcDNA3.0 and sequenced. Six plasmids that had high identities with the germ line genes were mixed to serve as **vaccines**. Eighteen Balb/c mice were divided randomly into three groups and were immunized respectively with the six mixed plasmids, the specific IgHV1 fragment of the Namalwa cell and the blank plasmid pcDNA3.0. 100 microg plasmid and 5 microg hIL-6 per mouse were injected into the muscle, 3 times in 4 weeks. Western blotting and indirect immunofluorescence staining were used to assess the humoral immune responses against the Namalwa cell. RESULTS: The specific humoral immune responses against the Namalwa lymphoma cell could be induced in both the IgHV1-mix group and the Na-IgHV1 group, and the antibodies could recognize the nature antigenic determinants in the surface of the Namalwa cell. The antibodies could be detected since the fourth week after the first immunization, and reached the climax at the sixth week. There was no significant difference of the titers of the antibodies between the two groups. The antibody couldn't be found in the negative control group. CONCLUSION: The mixed IgHV plasmids can be used as the universal nucleic acid **vaccine** against the **B-cell lymphoma** which expresses the same IgHV1 family genes.

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L13 ANSWER 35 OF 40 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 1998116668 MEDLINE
DOCUMENT NUMBER: 98116668 PubMed ID: 9455490
TITLE: Idiotypic **vaccine** for treatment of human **B-cell lymphoma**. Construction of IgG variable regions from single malignant B cells.
AUTHOR: Terness P; Welschof M; Moldenhauer G; Jung M; Moroder L; Kirchhoff F; Kipriyanov S; Little M; Opelz G
CORPORATE SOURCE: Institute of Immunology, University of Heidelberg, Germany.
SOURCE: HUMAN IMMUNOLOGY, (1997 Aug-Sep) 56 (1-2) 17-27.
Journal code: 8010936. ISSN: 0198-8859.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199802
ENTRY DATE: Entered STN: 19980224
Last Updated on STN: 19980224
Entered Medline: 19980211

AB Immunoglobulin idiotypes (Id) of malignant B cells represent highly specific markers which can be used for vaccination. PCR-amplification of immunoglobulin genes enables the rapid production of large amounts of Id **vaccines**. However, the separate amplification and subsequent recombination of heavy and light chains can lead to a loss of the relevant Id. To preserve the original chain pairs, we used single malignant B cells derived from an immunocytoma patient. Cytoplasm was extracted and the mRNA transcribed into cDNA. The VH and VL genes were then amplified by PCR and cloned into a vector for expression in E. coli. Id production was checked using an anti-Id mouse monoclonal Ab raised against the patient's tumor-specific IgG. One out of 3 constructs expressed the relevant Id. Analysis of the first 31 light chain residues revealed an identical sequence for the malignant B cells' IgG and the recombinant Id construct. Exchange of either the heavy or light chain with an unrelated chain resulted in loss of the Id. An unrelated sequence derived from the c-myc protein is coupled to the Id **vaccine**. The lymphoma patient was shown to have Abs to the c-myc sequence. This sequence therefore, increases the Id+ Ab's antigenicity. CD spectroscopy showed an alpha-helical structure for the c-myc epitope. In conclusion, a **B-cell lymphoma** autovaccine was produced containing immunogenic sequences that do not alter the steric conformation of the tumor-specific Id.